Supplemental Figure Legends

Supplemental Figure 1. The effectiveness of indolmycin to induce persistence is constrained by the availability of trp to *Chlamydia trachomatis.* HEp-2 cells were infected with *C. trachomatis* L2, and inclusion forming units (IFUs) were collected at 24 hpi and titrated on a fresh monolayer of HEp-2 cells in the absence of antibiotics. *C. trachomatis* was treated or not with 120 μM indolmycin at 10 hpi. At this time, DMEM was replaced with DMEM containing the denoted concentration of trp. A) Recoverable IFUs were quantified. Error bars represent the standard deviation between two biological replicates. B) Immunofluorescent images were taken at 24 hpi. All images were acquired on an AXIO Imager.Z2 with ApoTome.2 at 100x magnification. Scale bars represent 5 μm.

Supplemental Figure 2. Treatment with IFNγ and indolmycin or AN3365 have no additional morphological effects on *C. trachomatis*. HEp-2 cells were treated or not with IFNγ as described in the Materials and Methods. IFNγ treated samples had media replaced with IFNγ conditioned media at 10 hpi with or without 120 μM indolmycin or 1 μg mL^{-1} AN3365. Representative images are presented following fixation at 24 hpi and staining using primary antibody for MOMP. All images were acquired on an AXIO Imager.Z2 with ApoTome.2 at 100x magnification. Scale bars represent 5 μm.

Supplemental Figure 3. Electron micrograph images were collected to examine the morphological impact of 120 μM indolmycin and 1 μg mL^{-1} AN3365 on *C. trachomatis*. HEp-2 cells were infected with *C. trachomatis* at an MOI of 2.5 and treated or not with the denoted tRNA

synthetase inhibitor. Samples were collected and fixed at 24 hpi. Images were acquired on an FEI Tecnai G2 TEM operated at 80 Kv. Scale bars represent 2 μm, 500 nm, and 500 nm, respectively.

Supplemental Figure 4. Indolmycin and AN3365 do not affect host cell activity. PrestoBlue was used to measure cellular activity in uninfected HEp-2 cells after 24 hours of the specified treatment further detailed in the Materials and Methods. Student's T-test was used to compare background-corrected 570 nm absorbance values of each treated sample to an untreated control. * = P value < 0.05, ** = P value < 0.005, *** = P value < 0.0005.

Supplemental Figure 5. Trp deplete media induces an increase in *trpB*, but not *euo*, transcripts. HEp-2 cultures were infected with *C. trachomatis* at an MOI of 1. At 10 hpi, DMEM was replaced with standard DMEM (Untreated), DMEM lacking trp (No trp), or DMEM lacking trp with 120 μM indolmycin (Indolmycin). RNA transcripts were analyzed via RT-qPCR to compare the efficacy of trp deplete media in inducing persistence, as measured by increased *euo* transcript levels.

Supplemental Table 1. All primer sequences used for qPCR analysis of a given transcript.